presence of an intramolecular disulfide bond between two cysteine amino acids. Octreotate was prepared by an automated fluorenylmethoxycarbonyl (Fmoc) solid phase peptide synthesis using a commercial peptide synthesizer from Applied Biosystems (Model 432A SYNERGY Peptide Synthesizer). The first peptide cartridge contained Wang resin pre-loaded with Fmoc-Thr on a 25-µmole scale. Subsequent cartridges contained Fmoc-protected amino acids with side chain protecting groups for the following amino acids: Cys(Acm), Thr(t-Bu), Lys(Boc), Trp(Boc) and Tyr(t-Bu). The amino acid cartridges were placed on the peptide synthesizer and the product was synthesized from the C- to the N-terminal position according to standard procedures. The coupling reaction was carried out with 75 µmoles of the protected amino acids in the presence of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)/N-hydroxybenzotriazole (HOBt). The Fmoc protecting groups were removed with 20% piperidine in dimethylformamide.

After the synthesis was complete, the thiol group was cyclized with thallium trifluoroacetate and the product was cleaved from the solid support with a cleavage mixture containing trifluoroacetic acid water:phenol:thioanisole (85:5:5:5$^{v/v}$) for 6 hours. The peptide was precipitated with t-butyl methyl ether and lyophilized with water:acetonitrile (2:3$^{v/v}$). The peptide was purified by HPLC and analyzed by LC/MS.

Octreotide, (D-Phe-Cys'-Tyr-D-Trp-Lys-Thr-Cys'-Thr-OH (SEQ ID NO:2)), wherein Cys' indicates the presence of an intramolecular disulfide bond between two cysteine amino acids) was prepared by the same procedure as that for octreotate with no modifications.

Bombesin analogs were prepared by the same procedure but cyclization with thallium trifluoroacetate was omitted. Side-chain deprotection and cleavage from the resin was carried out with 50 µl each of ethanedithiol, thioanisole and water, and 850 µl of trifluoroacetic acid. Two analogues were prepared: Gly-Ser-Gly-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (SEQ ID NO:3) and Gly-Asp-Gly-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (SEQ ID NO:4).

Cholecystokinin octapeptide analogs were prepared as described for Octreotate without the cyclization step. Three analogs were prepared: Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2 (SEQ ID NO:5); Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:6); and D-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:7) wherein Nle is norleucine.

Neurotensin analog (D-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu (SEQ ID NO:8)) was prepared as described for Octreotate without the cyclization step.

Example 4

Synthesis of Peptide-Dye Conjugates (Figure 1B, n = 1, R_1 = Octreotate, R_2 = R_1 or OH)

The method described below is for the synthesis of Octreotate-cyanine dye conjugates. Similar procedures were used for the synthesis of other peptide-dye conjugates.

Octreotate was prepared as described in Example 3, but the peptide was not cleaved from the solid support and the N-terminal Fmoc group of Phe was retained. The thiol group was cyclized with thallium trifluoroacetate and Phe was deprotected to liberate the free amine. Bisethylcarboxymethylindocyanine dye (53 mg, 75 µmoles) was added to an activation reagent consisting of a mixture 0.2 M HBTU/HOBt in DMSO (375 µl), and 0.2 M diisopropylethylamine in DMSO (375 µl). The activation was

complete in about 30 minutes. The resin-bound peptide (25 µmoles) was then added to the dye. The coupling reaction was carried out at ambient temperature for 3 hours. The mixture was filtered and the solid residue was washed with DMF, acetonitrile and THF. After drying the green residue, the peptide was cleaved from the resin, and the side chain protecting groups were removed with a mixture of trifluoroacetic acid: water:thioanisole:phenol (85:5:5:5$^{v/v}$). The resin was filtered and cold t-butyl methyl ether (MTBE) was used to precipitate the dye-peptide conjugate. The conjugate was dissolved in acetonitrile:water (2:3$^{v/v}$) and lyophilized.

The product was purified by HPLC to give the monooctreotate-bisethylcarboxymethylindocyanine dye (Cytate 1, 80%, n = 1, R_2 = OH) and the bisoctreotate-bisethylcarboxymethylindocyanine dye (Cytate 2, 20%, n = 1, R_1 = R_2).

The monooctreotate conjugate may be obtained almost exclusively (>95%) over the bis conjugate by reducing the reaction time to 2 hours. This, however, leads to an incomplete reaction, and the free octreotate must be carefully separated from the dye conjugate in order to avoid saturation of the receptors by the non-dye conjugated peptide.

Example 5

Synthesis of Peptide-Dye Conjugates (Figure 1B, n=4 R_1 = octreotate, R_2 = R_1 or OH?)

Octreotate-bispentylcarboxymethylindocyanine dye was prepared as described in Example 4 with some modifications. Bispentylcarboxymethylindocyanine dye (60 mg, 75 µmoles) was added to 400 ul activation reagent consisting of 0.2 M HBTU/HOBt and 0.2 M of diisopropylethylamine in DMSO. The activation was complete in about 30